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Breaking the Code of Polyadenylation-Induced Translation

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The translation of many maternal mRNAs is regulated by dynamic changes in poly(A) tail length. During maturation of *Xenopus* oocytes, polyadenylation is mediated by three different *cis* elements in the 3′ untranslated region (UTR) of maternal mRNAs. In this issue, Piqué et al. (2008) explore the interplay of these elements to elucidate a combinatorial code that predicts the timing of polyadenylation and translation of maternal mRNAs.

Early animal development is programmed in part by mRNAs inherited by the egg at the time of fertilization. These silent maternal mRNAs are not translated en masse at one time or in any one cellular location; instead, their expression is often regulated both temporally and spatially. Although many maternal mRNAs are translated in the early embryo, some are translated even before fertilization, during the final phase of oocyte development known as oocyte or meiotic maturation. In immature oocytes arrested at the stage immediately preceding maturation, many silent mRNAs have short poly(A) tails; upon exposure of the oocytes to progesterone, which initiates maturation, the poly(A) tails grow and translation ensues. Polyadenylation and translation are not mere correlates of maturation but are required for this process. Therefore, at least some of the keys to understanding both translational control and meiotic progression would reside in the mechanism of cytoplasmic polyadenylation. In this issue, Piqué et al. (2008) describe a combinatorial code consisting of *cis*-acting elements in the 3′ untranslated region (UTR) of maternal mRNAs that predicts which mRNAs are repressed, which undergo polyadenylation and translation, and when these processes occur.

Most pre-mRNAs acquire long poly(A) tails in the nucleus, although those containing a cytoplasmic polyadenylation element (CPE) and nearby polyadenylation hexanucleotide AAUAAA (HEX) in their 3′UTRs undergo poly(A) shortening in the cytoplasm. Three of the most important proteins that control this process are CPE-binding protein (CPEB); poly(A)-specific ribonuclease (PARN), which deadenylates mRNAs; and Gld2, a poly(A) polymerase. Although PARN and Gld2 are both active, PARN activity is more robust; thus, the poly(A) is removed as soon as it is added by Gld2 (Barnard et al., 2004; Kim and Richter, 2006). However, a short poly(A) tail in and of itself does not necessarily repress translation; for this to occur, another factor, Maskin, is involved. Maskin not only binds to CPEB but also binds to the cap-binding factor eIF4E. This configuration of factors precludes the interaction of eIF4G with eIF4E and thereby inhibits translation by indirectly interfering with the positioning of the 40S ribosomal subunit at the end of the mRNA (Cao et al., 2006).

Following progesterone stimulation, the Aurora A kinase phosphorylates CPEB. CPEB phosphorylation causes CPEB to closely associate with cleavage and polyadenylation specificity factor (CPSF), the multisubunit complex associated with the HEX (Mendez et al., 2000a, 2000b), and leads to the expulsion of PARN from the ribonucleoprotein complex, resulting in Gld2-catalyzed polyadenylation of the mRNA (Kim and Richter, 2006). The elongated poly(A) tail is then bound by ePAB, an embryonic poly(A)-binding protein; ePAB subsequently interacts with and helps eIF4G to displace Maskin from eIF4E enabling translation to be initiated (Kim and Richter, 2007).

Hints of another level of regulation have come from studies showing that RNAs are polyadenylated at either early or late times of maturation. The late polyadenylation events require Mos kinase, a product of early polyadenylation-induced translation, as well as cyclin-dependent kinase 1(cdk1) and proteosome-mediated destruction of some but not all cellular CPEB (Mendez et al., 2002). In their current work, Piqué et al. have determined the features of mRNA 3′UTRs that dictate when polyadenylation will occur. They investigated the sequence requirements for CPEB-mediated translational repression and activation, and have generated algorithms that predict which, and to what extent, mRNAs are likely to be regulated by CPEB.

Xenopus oocytes contain five B type cyclins; the mRNAs encoding these proteins all contain CPEs, have short poly(A) tails, and are inactive. Moreover, these RNAs also contain a Pumilio-binding element (PBE), a sequence recognized by the Pumilio RNA-binding protein that has previously been shown to interact with CPEB (Nakahata et al., 2001). B1, B2, B4, and B5 become polyadenylated in response to progesterone, whereas B3 does not. Moreover, B1, B2, B4, and B5 do not become polyadenylated simultaneously; B1 and B4 are polyadenylated late in maturation and require partial destruction of the cellular pool of CPEB mediated by cdk1. B2 and B5, on the other hand, are polyadenylated early and are not affected by cdk1. A further examination of the degree of masking of each mRNA

revealed that B1, B4, and B5 were strongly repressed in oocytes, whereas B2 and B3 were not. In contrast, translational activation was greater for B1, B4, and B5 than for B2 or B3. What is responsible for these different types of regulation?

Piqué et al. next focused on the CPE, PBE, and HEX, the three features of UTRs that have been implicated in polyadenylation and/or translation. They generated a large set of 3′UTRs that varied according to the absence or presence of these elements, their distance from one another, the number of times they are present, and at least for the CPE, whether the sequence is consensus or nonconsensus—that is, whether or not it is an optimal binding site for CPEB. They then examined not only polyadenylation and translation in oocytes injected with these RNAs, but also used RNA gel shifts and UV-crosslinking analysis to define the relative binding characteristics of CPEB and Pumilio. Results from these experiments allowed the investigators to define a set of rules or a "combinatorial code" that dictates whether and when an mRNA will be regulated in maturing oocytes. First, they find that a single CPE, or 2 CPEs greater than 50 nucleotides apart, does not support translational repression even though polyadenylation still occurs. In this instance, the presence or absence of a PBE seems to have little effect. Second, repression requires two or more CPEs in fairly close proximity to one another, but these can probably be anywhere in a 3′UTR. Third, the PBE seems to augment repression when two or more CPEs are present. From these data, Piqué et al. infer that Maskin is recruited to or functions with a CPEB dimer but not with a CPEB monomer. In comparison the sequence elements directing polyadenylation appear to be simpler. A single CPE within 25 nucleotides of the HEX is optimal for polyadenylation; when the distance between these elements exceeds ~120 nucleotides, polyadenylation does not take place. A PBE can enhance the polyadenylation mediated by a single CPE; this and other observations suggest Pumilio helps to stabilize CPEB on the CPE.

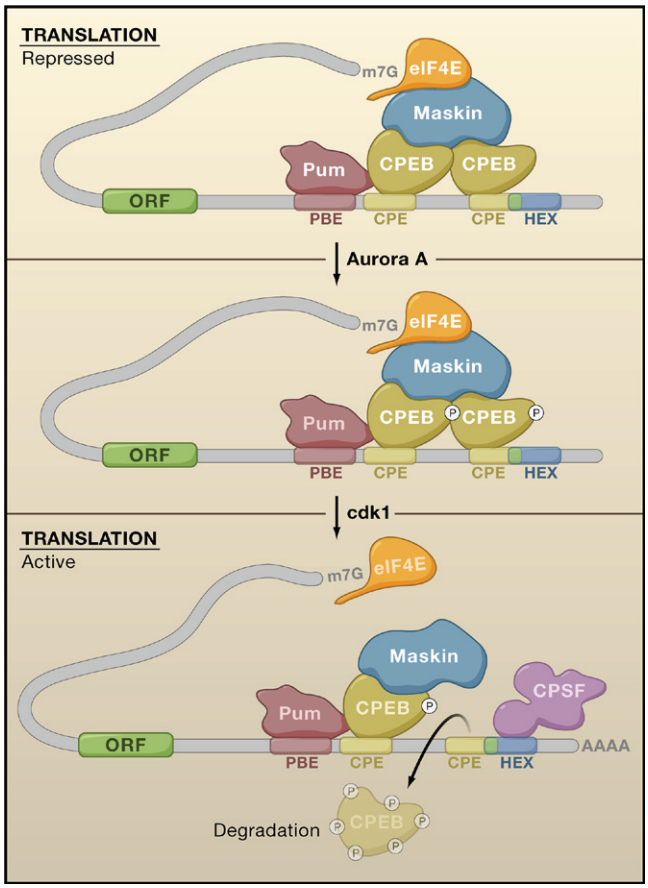

Figure 1. *cis* Elements Controlling Polyadenylation and Translation of Maternal mRNAs

One model for repression and activation of mRNA polyadenylation and translation in *Xenopus* oocytes. This mRNA contains two cytoplasmic polyadenylation elements (CPEs), each bound by CPE-binding protein (CPEB), and one Pumilio-binding element (PBE) bound by the Pumilio protein. In this example, one CPE overlaps with the polyadenylation hexanucleotide AAUAAA (HEX); CPEB could thus exclude the interaction of cleavage and polyadenylation specificity factor (CPSF) with HEX. The two CPEB proteins may be bound by Maskin, which in turn interacts with the cap binding factor eIF4E, thereby repressing translation. Following progesterone stimulation of Aurora A activity, both CPEB proteins are phosphorylated, which is soon followed by phosphorylation by cyclin-dependent kinase 1 (cdk1). These second modifications lead to ubiquitin-mediated destruction of CPEB. For some mRNAs the loss of CPEB binding allows CPSF to bind the HEX, which promotes subsequent mRNA polyadenylation. Although necessary, the precise function of CPSF in cytoplasmic polyadenylation is not clear.

What distinguishes mRNAs that are polyadenylated early from those that are polyadenylated late? The main determinant appears to be a CPE that overlaps with the HEX. That is, for RNAs that are polyadenylated early, Piqué et al. suggest that there must be no overlap between these two elements; hence, there would be no competition between CPEB and CPSF for RNA binding. For late adenylating mRNAs, there must be at least 2 CPEs, 1 of which overlaps with the HEX. In this way, CPEB association with both CPEs could exclude CPSF from binding the HEX, thereby inhibiting polyadenylation. Following cdk1 activation, CPEB is phosphorylated, and some gets destroyed (presumably stochastically), potentially leaving the HEX on some mRNAs available for binding by CPSF, which promotes subsequent polyadenylation (Figure 1).

Finally, Piqué et al. used computational methods to generate a long list of mRNAs that they predicted would be masked or translated according to their sequence codes; such predictions were borne out by experimental analysis. Interestingly, a large number of the mRNAs encode proteins involved in the cell cycle or differentiation, suggesting that CPEB is likely to play as important a role in embryogenesis as it does in maturing oocytes (e.g., Groisman et al., 2000; Mendez et al., 2002).

Xenopus oocytes have proven to be a rich source of material for investigating the kinase signaling events that lead to M phase progression (Turnquist and Maller, 2003). In a similar vein, oocytes have been valuable for analyzing RNA 3′ end processing and translational control; the study of Piqué et al. is one indication that the end of this tale is not yet in sight.

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Clearing the Path for Germ Cells

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The chemokine SDF-1a and its receptor CXCR4b guide germ cell migration in zebrafish by activating downstream signaling events. Boldajipour et al. (2008) now report that a second SDF-1a receptor, CXCR7, is also required for guided migration but does not function as a signaling receptor, and instead sequesters SDF-1a. These results highlight the importance of ligand clearance during guided cell migration.

Guided cell migration is central to processes ranging from embryogenesis to metastasis. Cells are guided by localized extracellular cues that serve as attractants or repellents. For example, zebrafish germ cells are guided by the chemokine SDF-1a (herein referred to as SDF-1) over hundreds of micrometers from their place of birth to the site of the future gonads. SDF-1 is expressed in somatic cells, whereas germ cells express the SDF-1 receptor CXCR4b (herein referred to as CXCR4). It is thought that the CXCR4-expressing germ cells are attracted by a local source of SDF-1. As the location of the SDF-1 source shifts during development, germ cells follow the source until they reach their final destination (Doitsidou et al., 2002; Knaut et al., 2003; Schier, 2003). In its simplest form, this model postulates that the spatially and temporally regulated expression of *SDF-1* mRNA generates local sources of SDF-1 protein that activate intracellular signaling events in CXCR4-expressing germ cells. Boldajipour et al. (2008) now provide evidence that the regulation of germ cell migration is more complex than anticipated. They find that SDF-1 sequestration by CXCR7—a second, recently described SDF-1 receptor—is also essential for germ cell guidance.

Intrigued by the discovery that CXCR7 is a receptor for SDF-1 (Balabanian et al., 2005; Burns et al., 2006), the authors characterized the role of CXCR7 during germ cell migration in zebrafish. Upon reduction of CXCR7 translation by morpholino antisense oligonucleotides, germ cells were scattered throughout the embryo instead of clustering at the site where the gonad develops. This phenotype resembled the germ cell guidance defect in CXCR4 mutants; however, in contrast to CXCR4, CXCR7 expression was not enriched in migrating germ cells but present at low levels throughout the early embryo. This expression pattern suggested that CXCR7—contrary to CXCR4—might not be required in germ cells for proper migration. Indeed, germ cells with reduced CXCR7 activity migrated normally when transplanted into wild-type embryos, whereas wild-type germ cells were misguided in embryos with reduced CXCR7 activity. Hence, CXCR7 is mainly required in somatic tissues surrounding the germ cells.

How does the activity of CXCR7 in somatic cells affect migration of neighboring germ cells? There are two simple models. First, CXCR7 signaling might generate a secondary signal that influences germ cell migration. For example, in response to CXCR7 signaling, somatic cells might produce adhesion molecules that influence the interaction of germ cells with their substrate. Alternatively, CXCR7 might not signal but directly alter SDF-1 activity. For example, CXCR7 might remove SDF-1 from the extracellular space. Consistent with previous studies (Burns et al.,